RISKS FROM MICROBIOLOGICAL AND CHEMICAL CONTAMINATION OF FISH MATERIALS

ABSTRACT: Fish is today one of the most important commercial material. In our market as in the world market they are present in fresh and manufactured products. Fish products always have some risk. They could be contaminated with dangerous chemicals and biological contaminants. From biological originated polutions 
Aflatoxin and other Mycotoxins are very dangerous. The contamination starts in fish ponds, in canals and swamps. Also, the contamination occurs at storage and preparation of fish materials. It is very important to recognize hygienically correct fish material and control fish food and different products from fish by educated inspectors (Vlahović, 1999).

KEY WORDS: Contamination, Fish food, Inspectors, Storage effect, Toxins

INTRODUCTION

The inspection program also encompasses the maintenance of databases on contaminants in fish and fish products and the inspection of fishing. Also, all consumers and trade complaints involving fish products are investigated to determine the cause of the complaint and the appropriate follow-up action.

Compared to European Union and many other countries, aquaculture in Serbia and Montenegro (SCG) is a relatively new industry. Until the 1980s, aquatic species were produced principally to provide seeds for restocking programs and family consumption. Although trout has been raised for food since early 1790s, only with the advent of commercial aquaculture of sardines, trout, carp, cat fish, perch, tolstolobic, sturgeon, hybrid striped bass and pike.¹ Others aquaculture species grown for food are shrimp, oyster, clam, mussels and crawfish (crayfish). To support this fast growing segment of agriculture, and

¹ Controlled production according to European and world standard has begun.
maintain its global competitiveness, several institutions as universities, governmental institutions for agriculture and fishing union. These institutions are actively engaged in research on nutrition, feeds and feeding of various commercially important fish species and other species of interest in aquaculture. The goals are to develop low-cost and environmentally-friendly feeds and more efficient feeding strategies, increase production efficiencies, improve fish health, decrease production cost, improve product quality and minimize environmental impact. The major research areas include nutrient requirements and their effects on stress and disease resistance, nutrient metabolism and nutritional physiology, development evaluation of nutritional value and nutrient bioavailability of alternative feed ingredients, effects of antinutritional factors and feed-borne toxins (mycotoxins) on fish growth, water quality, reproduction and fish health (Weibel, 1964).

Poisoning-algal toxins

Algae are important part of marine plankton serving as food for variety of aquatic animals. Algae are important factor of regeneration of water and treatment of water in fish pond. Algae may present variable morphology. Species can be unicellular as well as multicellular, some algae grow up to 20—30 meters of length. Algae serve as food for aquatic animals, they may be used as ingredients such as the production of agar agar, used in food, in drugs, as basis for bacteriological media and in many other ways. Chlorophyll is often hidden by yellow, brown, blue and red pigments. This gave the origin to a classification of algae in Xanthophyceae orRodophyceae. Algae produce starch, mannite, leucosin and oil. Classification of algae is often not up date, but some are didactical and will still be used even when there is a new classification proposed, so this is why the present classification proposed by Strasburger is used here. The plant kingdom is divided in 7 great divisions:

1. Bacterioiphyta containing bacteria,
2. Cyanophyta containing the blue-green alga,
3. Phycophyta containing all other alga,
4. Mycophyta containing mushrooms,
5. Bryophyta containing mosses,
6. Pteridophyta containing ferns,
7. Spermatophyta containing plants with seeds.

Algae which produce toxins are settled in division 2. Cyanophyta and division 3. Phycophyta, Flagellatae.
Division 2: Chroococcales, generally unicellular, forming jelly on rocks, Hormogonales (filaments)
Division 3: Chrysomonodale, Heterochloridales, Cryptomonodales, Dinoflagellatae, Euglenales, Protochloridale, and Volvoccales.

2 Start taking part in programmes and projects to solve the problems.
The *Flagellatae* have subdivision: *Chlorophytina* includes the green algae (*Chlorophyceae*), which live mainly in fresh water as plankton and form the green coating on rocks and the bark of trees. Other *Flagellatae* are: *Pyrrophytina, Euglenophytina, Rhaeophytina, Rhodophytina* and *Cyanophyta*.

**Algal toxins**

Beside useful algae, there are many single cell algae which produce toxins. These species develop rapidly under favourable conditions forming algae carpets in seawater killing fish. Marine animals such as oysters, crustaceae and different types of fish may eat toxic algae storing toxins. According to the species of algae, the symptoms of poisoning are (Wellee, 1931):

1. Damage of the nervous system, (Paralytical Shellfish Poisoning).
2. Loss of memory (Amnesic Shellfish Poisoning)
3. Neurotoxic phenomena (Neurotoxic Shellfish Poisoning)
4. Sodium channel blocking in nervous cells (Tetrodotoxin).

In summer the temperature of fish ponds, rivers, branches of river, lakes, chanel, swamps and seawater rises causing high growt of algae.

Damage of the nervous system: They are caused by toxins produced by *Dinoflagellata* such as *Alexandrium* spp. The Paralytical Shellfish Poisoning toxins are water soluble.

Damage of the digestive tract: Toxins of Diarrhetic Shellfish Poisoning are oedmic acid and the Dinophysis toxin which are liposoluble causing strong diarrhea.

Loss of memory: A poisoning with Amnesic Shellfish Poisoning which is caused by the dominoic acid (DA) of the alga *Nitzsca pungens*. This algae is found also in Europe. The maximum tolerable amount of dominoic acid in Germany is 20 mgDA/Kg in mollusc meat.

Neurotoxic phenomena: The neurotix Shellfish Poisoning toxins are produced by *Gymnodinium breve*, also denominated as *Ptychodiscus breve*. Amnesic Shellfish Poisoning toxins may be classified in to types: Brevetoxin A and Brevetoxin B.

Sodium channel blocking in nervous cells: *Tetradotoxin* is also called *Fugu-toxin*. It may be produced by some fish of the family of *Tetradontidae* (*Ta-kifugu* sp.). The *Tetradotoxin* blocks the sodium channel of nervous cells acting neurotoxic. This toxin has no absorption of ultra violet light and is not fluorescent.

*Saxitoxin*: is an algal toxin of the Paralytical Shellfish Poisoning type, being water soluble.

**Bacterial poisoning**

Bacteria can settle on food. Due to industrialisation and globalisation they can be widespread turning: endemic, epidemic and pandemic. The bacteria present in food can:
1. Spoil the food causing off-taste and off-smell.
2. Produce toxins under favourable conditions of growth, causing acute poisoning or subacute but very harmful alterations such as cancer.
3. Be infectious causing diarrhea and other serious diseases.
4. Be opportunists.

**Microorganism which spoil food**

Moulds, yeasts, *Escherichia coli* and *Proteus* can produce toxins when present in food and having sufficient time during storage under appropriate temperatures. In this case, the microorganisms do not necessarily need to be alive when reaching the final consumer. *Clostridium botulinum* produces exotoxins from type A, B, C, D, E and F. They are the strongest toxins which are known and act as neurotoxins. They inhibit the excretion of *acetylcholine* avoiding thus the transmission of signals from the nerve to the muscle causing paralysis comparable to the effect of *curare*. The endotoxins which are thermo unstable are formed in canned food with a pH higher than 4.5 and about 6 months of storage. This toxin is destroyed when food is cooked before serving. *Escherichia coli* produces an enteroxin under bad hygienic conditions. *Salmonella enteritides* produces a heat unstable exotoxin mainly in ground in eggs in poultry in milk powder, in chocolate, in fish meat and fish salads. *Shigella dysenteriae* and *Shigella sonnei* produce endotoxin or heat unstable exotoxins. *Shigella dysenteriae*, *Shigella sonnei* and *Staphylococcus aureus*, produce thermostable toxins. The toxins produced by *Staphylococcus aureus*, can be classified serologically as toxins A, B, C1, C2, D, E and F.

All microorganisms cited as the producers of toxins are able to cause infections. The microorganisms must be alive and in sufficient number to cause an infecton.

**Mycotoxins**

Mycotoxins are poisonous metabolites of certain moulds which can cause pathological changes in humans and animals. The most important species which produce mycotoxins are *Aspergillus*, *Penicillium* and *Fusarium*. Intoxication takes place through ingestion of contaminated food more seldom by inhalation or skin resorption. Mycotoxins, unlike the bacterial or algal toxins, generally do not produce acute intoxication but they are known as strong cancerogenic, teratogenic with chronic activity. Direct contamination with mycotoxins can take place when moulds grow on food. Indirect contamination can take place when mycotoxins which contaminated feed are ingesteded by cattle and pork. Milk, eggs and fish meat are examples of indirect contamination of food caused by spoiled feed containing *Aflatoxins, Ochratoxin A* and some of the *Fusaria toxins*. The direct contamination caused by moulds growing on food is of great importance on cereals, oil seeds, coffee, fruits, vegetables, spices, some types of cheese, like roquefort cheese, and fish meat products with wheat flour, corn germ and rice (Gasztónyi, 1979).
Aflatoxins

Aflatoxins are mycotoxins which are exclusively produced by the mould Aspergillus flavus and Aspergillus parasiticus (Radmilović, 2002). Important are Aflatoxins B1, G1 and G3. Aflatoxin B1 is the strongest cancerogenic compound known. It causes liver cancer. In food, Aflatoxin M1 is sometimes present and is almost as poisonous as Aflatoxin B1. In fish feed in fish pond, the most frequent Aflatoxin is B1 being often found together with B2, G1, G2 (Fishbein, 1972).

Fusaria toxins

Fusaria toxins is a generic term for the so-called fade-toxin produced by Fusaria moulds which produce wrinkling of plant parts (Bamburg, 1968).

Ochratoxin A

Ochratoxin A is a mycotoxin produced by moulds of genus Penicillium and Aspergillus. It is a water soluble cumarin derivate, cereal, coffee, spices and other fish food (in fish pond). The growth of moulds and production of Ochratoxin A is speeded by high temperatures and high moisture during harvest, handling, drying, storage and transport. Ochratoxin A is carcinogenic and genotoxic in mice and rats. Heating during cooking and backing does not inactivate Ochratoxin (Scott, 1965). Stored cereals can be decontaminated with an atmosphere of 2% NH3 at 20 degrees during 4 to 6 months.

Poison of heated foods

Heterocyclic aromatic amines are caused by heating protein rich foods as fish meat. They are carcinogenic. Polycyclic aromatic carbon and polycyclic aromatic hydrocarbon are formed when fat drips from grill fish meat and are brought back with smoke (smoked salmon, herring and tuna) and flames contaminating the foods. They are carcinogenic.

CHEMICAL AND BIOLOGICAL ANALYSIS OF TOXINS

For the analysis of different paralytic toxins it is recommended the ionic-pair chromatography with RP-C18 column. High Pressure Liquid Chromatography (HPLC) is used as analytical method of Amnesic Shellfish Poising an RP-C18 column without derivatisation. Dominoinic acid down to 1,0 mg/kg in mollusc meat can be detected with derivisation before the column with fluorenilmetoxicarbonilchlorid. The leading world standardized methods for Neurotic Shellfish Poisoning analysis are HPLC-MC and immunoassays.
Saxitoxin is extracted from acid solution and after extraction purified with periodic acid in alkaline medium. Saxitoxin is then read fluorometrically against a standard curve. Standard solution: Saxitoxin dissolved in acetic acid 0.1 cm³/dm³. Further dilution are made with sulphuric acid in a way that 2 cm³ of the dilution are added to 2 cm³ NH₄OH and 0.1 cm³ 0.1 mol/dm³ periodic acid. Reading made at 388 nm.

Sample with Saxitoxin purified in column of 1.0 cm diameter, is charged with approximately 5 g resin which gives a length of 5 cm, is then washed with 100 cm³ buffer of potassium acetate at a pH 5.2, followed with 50 cm³ distilled water. Saxitoxin is then eluted with sulfuric acid 0.5 mol/dm³ until 20 cm³ are obtained in a volumetric flask. The velocity of elution should not exceed 3 cm³/min. After 15 minutes 0.2 cm³ of glacial acetic acid are added to the solution and read against a blank containing the same components as before having periodic acid changed with water. With the same methods the mycotoxins are analysed.

The known fresh water fish species

In Novi Sad areas including National park Fruška Gora in fishing there occur the following fish species:

— Omnivora fishes: Barbel (Barbus barbus), ide, orfe (Leuciscus idus), Vimba vimba, clamp (Chondrostoma nasus). Fresh water carp (Pelecus cultratus), robin (Scardinius erythropthalmus), Rutulus rutilus, Carassius auratus, carp (Carassius carassius), tench (Tinca tinca), gudgeon (Gobio gobio), loach (Misgurnus fossilus), Carassius carassius, Cyprinus carpio, carp (Cyprinus carpio), sturgeon (Acipenser ruthenus), bream (Abramis brama), Blicca bjoerka, Abramis sapa, Abramis ballerus, bleak (Alburnus alburnus), Rhodeus sericeus amarus, Pseudorasbora parva.

— Herbivora fishes: Stenopharingodon idella, Hypoptamichthys molitex, Aristichtis nobilis.

— Predators: Eel (Anguilla anguilla), perch (Stizostedion lucioperca), Stizostedion volgensis, pike (Esox lucius), catfish (Silurus glanis), burbot (Lota lota), Ictalurus nebulosus, Aspius aspius, klena (Leuciscus cephalus), Zingel zingel, Micropterus salmoides, perch (Perca flaviatilis, Lepomis gibbosus).

DISCUSSION

This paper is for the purpose of the education of sanitary inspectors and in schools. There was included in the questionnaire 337 pupils of “Prva vojvodanska brigada” elementary school in Novi Sad, 13—14 years of age. The questionnaire deals with indirect poisoning with toxins in fish food. In summer, when in fish ponds the oxigen level is decreased, the bacteria liberate toxins are in dangerous quantity. Also the green algae develop toxins. The most dangerous is the poisoning with inadequate stored fish food. It has also to be no-
ted that the poising with fish has storage effect. The mentioned problems are included in five quetions. The questions given below had interesting answers.

1. When do you consume fish food? Answer: never (15), rare (41), 1—2 times monthlly (119), one or two times weakly (141) and very often (21).

2. Where is fish purhased for your house? Answer: In the restaurant (8), in fish market (124), in the shops (108), at private sellers (57), fishing by oneself (37) and not interested (3).

3. Which kind of fish do you purchase? Answer: Frish (89), frozen (130), various statue (104), do not purchase (7) and not informed about (7).

4. How the consumed fish food is prepared? Answer: cooked (36) baked (101), on oil roasted (90), breaded (92), not preparared (7), smoked fish (4) and from can (7).

5. What do they do with fish food which remain after consuming? Answer: they eat it later cold (125), throw away (88), heat it again (76) and no rest (48).

— From the first question it is seen that many of then eat fish.
— The second question shows that 28% of fish food come from risky origins and sellers.
— The third question gave answer that people purchase mostly frozen fish which include many uncertainities including technology and refrozen processes.
— From the answers to the fourth question it is seen that 55% of the prepared fish food was done in risky way.
— The fifth answer shows that 37% eat the rest fish food cold, which is a great risk like again heated food which is 23%.

LITERATURE


РИЗИЦИ ОД МИКРОБИОЛОШКИХ И ХЕМИЈСКИХ КОНТАМИНАТА У РИБЉЕМ МАТЕРИЈАЛУ

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Резиме

Рибљи производи су данас на светском тржишту веома ценjeni. Они у процет улазе као свежа риба или као прерађенине. Међутим, сви рибљи производи носе у себи опасност, пре свега од трошивања. Само рибе се често контаминирају хемијским и биолошким загађивачима вода и рибље хране. Контаминација је најизраженија у рибињацима, каналима и у барама, као што је у окolini Новог Сада Петроварадинско-кowiљски рит. Одређене врсте алге или укварена, буђава храна за рибе су највећа опасност за контаминацију. У њиховим организмима водичи и друге опасне материје се постепено гомилази и кумултивним ефектом додатно угрожавају потрошача рибљег материјала. Грабљивице и рибе свештоједи још више кумулирају у себе токсине и друге токсичне супстанце. Начин спремања и складиштења јела од риба исто тако треба убраjати у потенцијалну опасност од загађивања од токсина. Нарочито се то односи на поховане рибе, затим са брашном и пиринчем припремљене рибле специјалитете. Важно је, за- то, првенствено едукувати тржишне инспекторе како би они преузели потребне превентивне мере.